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SCIENTIFIC NOTE

LABORATORY EVALUATION OF TOXICITY OF 16 INSECT REPELLENTS IN AEROSOL SPRAYS TO ADULT MOSQUITOES[1]

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ABSTRACT. Sixteen commercial insect repellents (6 botanical and 10 synthetic organic products) in spray formulations were evaluated in the laboratory for adult knockdown (KD) and mortality of laboratory-reared female *Aedes aegypti, Aedes albopictus,* and *Anopheles quadrimaculatus.* All tested products produced significant posttreatment KD and 24 h mortality of all 3 mosquito species. In our study, the synthetic organic repellents induced faster KD and KD of higher magnitude in adult mosquitoes than the botanical product repellents except geraniol-based MosquitoSafe™. All tested formulations except 2 botanical repellent products caused 100% 24-h mortality of *Ae. aegypti* and all but 1 caused 100% 24-h mortality of *Ae. albopictus* and *An. quadrimaculatus.*

KEY WORDS Insect repellents, toxicity, *Aedes aegypti, Aedes albopictus, Anopheles quadrimaculatus,* mosquito adulticides

Prevention of mosquito bites and mosquitoborne diseases through the use of insect repellents is one component in an overall mosquito management strategy. Presently, numerous insect repellent products are available commercially in a variety of formulations. Some of these products contain active ingredient(s) that are botanical and some are synthetic organic products, with a vast majority available as sprays because this formulation is easy to use indoors or outdoors. Although the available repellents are primarily used for repelling nuisance and vector insects, little information is available on other types of activity of these products, such as insect knockdown (KD) and mortality, except for ingredients of Neem Aura™ and permethrin (ingredient of Repel® Permanone). While testing for repellency of commercially available repellents against mosquito bites in a recent laboratory study, we observed mortality in adult mosquitoes shortly after exposure to some repellents. This prompted us to select and test a number of commercially available insect repellent products in spray formulations for KD and mortality activity against females of 3 species of laboratory-colonized mosquitoes. This information may be useful for understanding the toxicity of repellents against adult mosquitoes and possibly for extending the use of repellents for mosquito control purposes.

The mosquito species used in our tests were *Aedes aegypti* (L.), *Aedes albopictus* Skuse, and *Anopheles quadrimaculatus* Say. All mosquitoes were reared in the laboratory by methods described by Gerberg et al. (1994). Each species was maintained in the laboratory free of exposure to insecticides or repellents. Mosquitoes were 5- to 7-day-old, unfed (no blood meal) females when treated.

Sixteen commercially available insect repellent products, 6 that contain botanical active ingredients and 10 that contain synthetic organic active ingredients, were tested (Table 1). Eight products in the synthetic group contained deet (*N,N*-diethyl-3-methylbenzamide) as the active ingredient.

Paper cans (17 cm in diameter and 17 cm high) (The Fonda Group Inc., St. Albans, VT) were modified for use as test cages in these evaluations. The open end of the can was covered with a nylon screen (1.7-mm mesh) held in place with a rubber band. A small hole (1.5-cm diameter) was made on the side of the can near the bottom for introducing adult mosquitoes. Fifteen laboratory-reared female mosquitoes were transferred into the cage with a mouth aspirator and the hole was plugged with a stopper. Mosquitoes were allowed to acclimate in the cage for 1 h before treatments. Each treatment was applied through the screen of the cage by holding the spray outlet 15 cm from the screen top and releasing the spray into the cage by using 1 squirt (0.5 ml). The use of 1 squirt in these evaluations was based on an initial laboratory screening of some of the test products against *Ae. albopictus* by using 1, 2, 3, 4, and 5 squirts, where 1 squirt produced almost 100% adult mortality 24 h after exposure. Mosquito KD was recorded at 1, 5, 15, 30, and 60 min after treatment; adults surviving >60 min were provided with sugar water in pads of absorbent cotton placed on the screen top of each cage and observed 24 h later for mortality. Each

[1] This paper reports the results of research only. Mention of repellent, commercial, or proprietary product does not constitute a recommendation or endorsement of this product by the U.S. Department of Agriculture.

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Table 1. Product name, active and inert ingredients with their concentration, and name and address of manufacturers of insect repellent products tested in the laboratory against 3 species of laboratory-reared mosquitoes.

Product name	Ingredients and concentrations	Manufacturer or company
	Botanical insect repellent products	
GonE!	PABA (*p*-aminobenzoic acid), natural alcohol, vegetable glycerin, soybean oil, organic Aloe Vera®, lavender oil, eucalyptus oil, menthol, camphor, rosemary and sage oils	Aubrey Organics, Tampa, FL
MosquitoSafe™	Geraniol 25%, mineral oil 74%, aloe vera 1%	Not available
Natrapel™	Citronella 10%, inert ingredients 90% (water, xanthan gum, lauryl sulfate, potassium sorbate, citric acid)	Tender Corp., Littleton, NH
Neem Aura™	Aloe vera, neem leaf extract, ethyl alcohol (from grain), vegetable glycerin, coconut oil, decylpolyglucose (from coconut), neem oil, myrrh extract, lemongrass oil, orange oil, citronella oil, lavender oil, anise oil, cedarwood oil, rhodiumwood oil, barberry extract, thyme extract, goldenseal extract, chamomile extract	The Original Neem Co., Neem Aura Naturals Inc., Alachua, FL
Skin-So-Soft Bug Guard	Citronella oil 0.1%, inert ingredients 99.9%	Avon Products Inc., New York, NY
SunSwat	Octyl methoxycinnamate, octyl salicylate, benzophenone-3, isopropyl palmitate (palm oil), dimethicone (silica derived), cyclomethicone (silica derived), PVP (polyvinyl pyrrolifone)/eicosene copolymers (waterproof agents), essential oil blend of citronella, bay, cedarwood, lavender, vetivert, patchouli, juniper, tea tree, lemon peel, pennyroyal, tansy, goldenseal, propylparaben	Kiss My Face Co., Gardiner, NY
	Deet insect repellent products	
Off! Skintastic®	Deet 6.65%, related isomers 0.35%, inert ingredients 93%	S.C. Johnson & Sons Inc., Racine, WI
Cutter® Unscented Insect Repellent	Deet 9.50%, other isomers 0.5%, inert ingredients 90%	United Industries Corp., St. Louis, MO
Off!® Unscented	Deet 14.25%, related isomers 0.75%, inert ingredients 85%	S.C. Johnson & Sons Inc., Racine, WI
Family Formula Repel® Insect Block™	Deet 14.25%, other isomers 0.75%, inert ingredients 85%	Wisconsin Pharmacol Co. Inc., Jackson, WI
Bugout™ Insect Repellent	Deet 14.25%, other isomers 0.75%, inert ingredients 85%	IQ Products Co., Houston, TX
Eckerd Insect Repellent	Deet 14.25%, other isomers 0.75%, inert ingredients 85%	Eckerd Drug Co., Clearwater, FL
Off!® Deep Woods	Deet 23.80%, related isomers 1.2%, inert ingredients 75%	S.C. Johnson & Sons Inc., Racine, WI
Ultra Muskol	Deet 38.00%, other isomers 2%, inert ingredients 60%	Schering-Plough Health Care Products Inc., Mississauga, ON, Canada
	Other synthetic organic insect repellent products	
Autan®	Aqua, alcohol denatured, hydroxyethyl butyl piperidine carboxylate, citric acid, perfume	Bayer Ltd., Dun Looghaeire Co., Dublin, Ireland
Repel® Permanone	Permethin 0.5%, inert ingredients 99.5%	Wisconsin Pharmacol Co. Inc., Jackson, WI

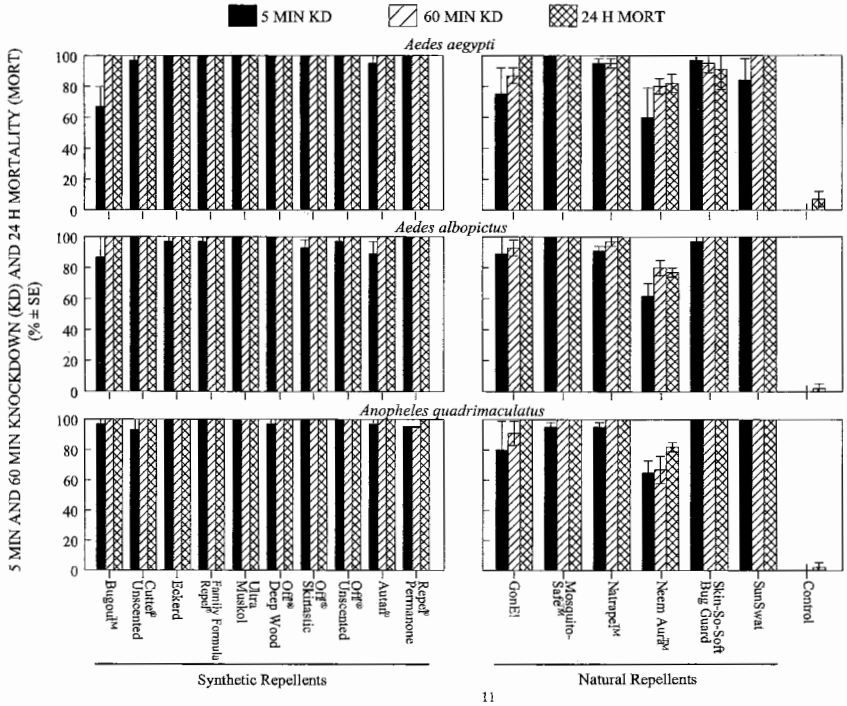

Fig. 1. Knockdown and adulticidal activity of synthetic organic and botanical repellents against laboratory-reared female *Aedes aegypti, Aedes albopictus,* and *Anopheles quadrimaculatus* exposed to repellents in modified paper can test cages in the laboratory.

treatment was replicated 3 times for each species. The control cages were sprayed with 0.5 ml of acetone and provided with sugar-water pads. Mosquito KD and mortality responses were analyzed by a multiple-way analysis of variance.

In this study, the 50% knockdown dose (KD_{50}) of the test products could not be determined because 1 squirt (0.5 ml) of a product resulted in KD of most of the adults. Consistently smaller amounts of the test products, in less than 1 squirt, were not possible to obtain by using the commercial spray cans. Similarly, the KD time for 50% of the adults (KT_{50}) values for the dose of each tested product were not available even at 1 min after treatment because they were <1 min in most cases.

The 5-min KD, 60-min KD, and 24-h mortality data for the synthetic and botanical repellents are summarized in Fig. 1. Because the 1-min KD data for all test mosquito species were very similar to the 5-min KD data, and 15- and 30-min KD data were similar to 60-min KD data, only 5- and 60-min KD data for each mosquito species are provided.

The following products did not cause 100% KD at 5 min after treatment: Bugout™ and Cutter® Unscented, against *Ae. aegypti*; Bugout, Eckerd, Family Formula Repel, Off!® Skintastic, and Off! Unscented, against *Ae. albopictus*; and Bugout, Cutter Unscented, and Off! Deep Woods, against *An. quadrimaculatus.* Autan® was less effective at 5 min against all 3 mosquito species compared with Repel Permanone, which caused 95% KD of *An. quadrimaculatus* at 60 min after treatment (Fig. 1).

Among the repellents containing botanical products, Neem Aura was the least effective against all 3 mosquito species, followed by GonE! (Fig. 1). MosquitoSafe™ and SunSwat were the most effective in causing mortality of adult mosquitoes. In general, all botanical product repellents, except for Neem Aura and Skin-So-Soft Bug Guard against *Ae. aegypti,* and Neem Aura against *Ae. albopictus* and *An. quadrimaculatus,* caused 100% mortality at 24 h after treatment.

All test products provided significant KD and mortality in all 3 mosquito species compared with controls ($F = 51.746$; df = 15, 60; $P < 0.001$); % KD also increased significantly with exposure time ($F = 46.173$; df = 2, 60; $P < 0.001$). No differences among species were noted among treatments for either KD or adult mortality ($F = 0.770$; df = 2, 60; $P > 0.05$). However, significant differences were found between treatments with botanical repellents ($F = 29.634$; df = 7, 40; $P < 0.01$) where Neem Aura caused significantly less 24-h mortality and GonE! caused significantly lower 60-min KD than the other botanical repellents tested.

Except for the report of Sarkaria and Brown (1951) showing mortality response of female *Aedes* to liquid repellent compounds, no detailed reports have been made in the literature on the adulticidal activity of mosquito repellent products. In a recent study, Xue et al. (2001a, 2001b) showed that selected insect repellents not only deter mosquito oviposition, but also are potent mosquito larvicides. Thus, insect repellents, in addition to providing protection from mosquito bites, may be useful in mosquito control. The present study also demonstrated the usefulness of modified paper cans for testing mosquito adulticides. They are simple to use and provide an alternative to the World Health Organization susceptibility test kits (WHO 1986), and the use of insecticide-coated vial bioassay techniques (Robert and Olson 1989) or wind tunnels (Mount et al. 1976).

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